BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Problem Image Mailbox.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 May 2001 (25.05.2001)

PCT

(10) International Publication Number
WO 01/36321 A1

(51) International Patent Classification[7]: B81C 1/00, B82B 1/00, 3/00, B01D 63/00

(21) International Application Number: PCT/US00/31749

(22) International Filing Date:
17 November 2000 (17.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/166,049 17 November 1999 (17.11.1999) US

(71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 1111 Franklin Street, Fifth floor, Oakland, CA 94607-5200 (US).

(72) Inventors: HANSFORD, Derek; 2719 Quarry Vally Road, Columbus, OH 43204 (US). FERRARI, Mauro; 8189 Crossgate North Court, Dublin, OH 43017 (US).

(74) Agents: GALLIANI, William, S. et al.; Pennie & Edmonds LLP, 1155 Avenue of the Americas, New York, NY 10036 (US).

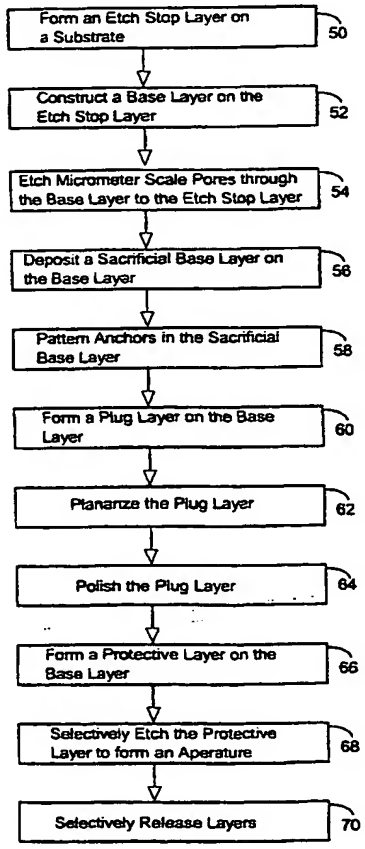
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

[Continued on next page]

(54) Title: APPARATUS AND METHOD FOR FORMING A MEMBRANE WITH NANOMETER SCALE PORES



(57) Abstract: A method of forming a membrane with nanometer scale pores includes forming a sacrificial etch stop layer on a substrate. A base layer is constructed on the sacrificial etch stop layer. Micrometer scale pores are formed within the base layer. A sacrificial base layer is built on the base layer. The sacrificial base layer is removed from selected regions of the base layer to define nanometer scale pores within the base layer. The resultant membrane has sub-fifty nanometer pores formed within it.

WO 01/36321 A1

— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

APPARATUS AND METHOD FOR FORMING A MEMBRANE WITH NANOMETER SCALE PORES

This application claims priority to the U.S. Provisional Patent Application entitled, "Apparatus and Method for Forming a Membrane with Nanometer Scale Pores," Serial Number 60/166,049, filed November 17, 1999.

BRIEF DESCRIPTION OF THE INVENTION

This invention relates generally to membranes with nanometer scale pores that may be used in filtering applications. More particularly, this invention relates to the use of microfabrication processing techniques to form nanometer scale porous membranes.

BACKGROUND OF THE INVENTION

There is a revolution occurring in biological research. Emphasis is rapidly shifting towards the view of biology in terms of a complex series of physical and chemical interactions, and interdisciplinary research between engineers, biologists, physicists, and clinicians is becoming prevalent. A rapidly developing field of research is the use of micro-fabrication to make mechanically, electrically, and/or chemically interactive structures for biological research and applications, known collectively as BioMEMS, with the term "Bio" referring to biology, and the term "MEMS" referring to MicroElectroMechanical devices. By using semiconductor based micro-fabrication techniques, MEM structures can be fabricated with spatial features from the sub-micron range up to several millimeters. These multi-scale structures correspond well with hierarchical biological structures, from proteins and sub-cellular organelles to the tissue and organ levels. This structural correlation allows scientists to investigate biological structure on their respective size scales and

interact in more appropriate and responsive manners to the structures within the body and within biological fluids.

Conceivably, it would be desirable to use standard micro-lithography to produce structures that can be used for basic biological research, diagnostic, and therapeutic applications. However, conventional lithographic techniques have feature size limitations that prevent their use for fabricating structures that can physically interact with molecules of biological interest, such as proteins, nucleotides, and various physiological nutrients. To interact directly with these molecules, features must be fabricated with sizes less than 50 nm, which is not projected for state of the art lithography until the year 2008. Furthermore, because of the fabrication techniques which are used for MEMS structures and the potential for contamination they introduce, state of the art equipment will not be used to fabricate these structures, leading to a further delay in the fabrication of direct interaction structures.

In view of the foregoing, it would be highly desirable to provide a technique for fabricating membranes with features less than 50 nanometers. Ideally, such a technique would rely upon standard lithography processing techniques and would yield a device that is compatible with biological research, diagnostic, and therapeutic applications.

SUMMARY OF THE INVENTION

The invention includes a filter comprising a membrane of elemental silicon with sub-fifty nanometer pores formed within it. The membrane has a glucose diffusion test result of at least 1 mg/dl and an albumin diffusion test result of at most 0.1 g/dl. The filter has a substrate, a buried sacrificial etch stop layer positioned on the substrate, with the membrane positioned on the buried sacrificial etch stop layer. In one embodiment, the buried sacrificial etch stop layer is silicon nitride.

The method of the invention includes forming a membrane with nanometer scale pores. A sacrificial etch stop layer is formed on a substrate. A base layer is constructed on the sacrificial etch stop layer. Micrometer scale pores are formed within the base layer. A sacrificial base layer is built on the base layer. The sacrificial base layer is removed from selected regions of the base layer to define nanometer scale pores within the base layer.

BRIEF DESCRIPTION OF THE DRAWINGS

For a better understanding of the invention, reference should be made to the following detailed description taken in conjunction with the accompanying drawings, in which:

FIGURE 1 illustrates a substrate with a sacrificial buried layer formed thereon in accordance with an embodiment of the invention.

FIGURE 2 illustrates a base layer formed on the sacrificial buried layer of Figure 1.

FIGURE 3 illustrates etched micrometer pores formed within a base layer and stopped by a sacrificial buried layer in accordance with the invention.

FIGURE 4 illustrates the deposition of a sacrificial base layer in accordance with an embodiment of the invention.

FIGURE 5 illustrates anchors formed in the sacrificial base layer utilized in accordance with the invention.

FIGURE 6 illustrates a plug layer formed in accordance with an embodiment of the invention.

FIGURE 7 illustrates the plug layer after mechanical polishing in accordance with an embodiment of the invention.

FIGURE 8 illustrates a protective layer and resultant selective etching utilized in accordance with an embodiment of the invention.

FIGURE 9 illustrates a fully released nanometer scale membrane after removal of the protective layers, and selective regions of the sacrificial buried layer.

FIGURE 10 illustrates processing steps used to construct the devices of Figures 1-9.

FIGURE 11 illustrates a device used to test the membrane of the invention.

FIGURE 12 illustrates glucose diffusion through three different nanopore membranes.

FIGURE 13 illustrates diffusion of glucose and albumin through micromachined nanopore membranes.

FIGURE 14 illustrates glucose diffusion through micromachined membranes incubated in pure glucose and mixed glucose/albumin solutions.

FIGURE 15 illustrates diffusion through millipore membranes incubated in pure glucose and mixed glucose/albumin solutions.

FIGURE 16 is a table illustrating diffusion of Albumin through various membranes.

Like reference numerals refer to corresponding parts throughout the drawings.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relies upon many prior art techniques in forming a membrane with nanometer scale pores. However, the invention also departs from the prior art in several key respects. These departures from the prior art facilitate the formation of pores less than 50 nanometer.

First, the technique of the invention relies upon a buried sacrificial etch stop layer. By way of the example, the buried sacrificial etch stop layer may be silicon nitride. The buried sacrificial etch stop layer operates as an etch stop, and is then removed to expose the nanopores of the invention.

The invention's use of a buried sacrificial etch stop layer as an etchant stop during the formation of nanometer scale pores is believed to be novel. While buried etch stop layers are used for structural purposes in the prior art, it is not believed that the prior art shows or suggests the formation of a buried etch stop layer, which operates as an etchant stop during the formation of pores, and which is subsequently etched away to expose pores.

The buried sacrificial etch stop layer facilitates three-dimensional control of the pore structure. Prior art techniques endeavored to control pore structure by balancing the etching of two different layers. The buried sacrificial etch stop technique of the invention facilitates the formation of pores less than 50 nanometers. Moreover, these pores can be uniformly formed across the entire wafer.

The buried sacrificial etch stop layer of the invention eliminates the prior art use of diffused boron. When diffused boron is used as an etch stop it provides an imprecise membrane depth. In addition, boron introduces stresses into the completed membrane.

Advantageously, the buried sacrificial etch stop layer of the invention provides absolute etching selectivity, as the layer will not be etched at all by the disclosed KOH etchant. In contrast, boron will be minimally etched in the presence of a KOH etchant.

The technique of the invention departs from prior art techniques in another important manner. Namely, the technique of the invention relies upon planarization of the outer structural layer to expose the total pore area, instead of the prior art approach of etching entrance holes in the top layer.

Preferably, the first step in the fabrication protocol is to etch a support ridge structure into a substrate. This is accomplished by simply etching a ridge structure prior to the deposition of the etch stop layer. The ridge provides mechanical rigidity to the subsequently formed membrane structure.

The buried sacrificial etch stop layer is then deposited on the substrate. For example, a low stress silicon nitride (LSN or nitride) is deposited on the substrate using low pressure chemical vapor depositions (LPCVD). In one embodiment, 0.4 μm of silicon nitride was used. The resultant structure is shown in Figure 1. Figure 1 illustrates a substrate 20 with a sacrificial etch stop layer 22 formed thereon.

The base structural layer (base layer) of the membrane is deposited on top of the stop layer 22. Because the stop layer 22 is thin, the structural layer is deposited down into the support ridges formed in the substrate 20. In one embodiment, 5 μm of polysilicon is used as the base layer. Figure 2 illustrates the base layer 24 positioned on the stop layer 22. Low stress silicon nitride may also be used as the base layer, in which case it operates as its own etch stop layer.

The next processing step is to etch holes in the base layer 24 to define the shape of the pores. Prior art masks may be used to define the pores. For example, the holes may be etched through the polysilicon by chlorine plasma, with a thermally grown oxide layer used as a mask. In this step, it is important to make sure the etching goes completely through the base layer 24, so a 10-15% overetch is preferably used. It is useful to note that the buried sacrificial etch stop layer 22 acts as an etch stop for the plasma etching of a silicon base layer 24. Otherwise, if the plasma punched through the etch stop layer, tighter control of the etch step layer would have to be exercised to prevent the complete removal of the nitride under the plug layer (to prevent removal in the final KOH etch). Figure 3 illustrates the result of this processing. In particular, the

figure illustrates holes 26 formed in the base layer 24, but terminating in the buried sacrificial etch stop layer 22. At this stage, the holes 26 define micrometer scale pores.

Pore sacrificial oxide is subsequently grown on the base layer 24. Figure 4 illustrates a sacrificial oxide 28 positioned on the base layer 24. This sacrificial oxide 28 is also referred to as a nanometer scale sacrificial base layer or sacrificial base layer. This sacrificial base layer 28 is used to define nanometer scale pores.

The thickness of the sacrificial base layer 28 determined the pore size in the final membrane, so control of this step is critical to reproducible membranes. This is accomplished by the thermal oxidation of the base layer 24 (e.g., a growth temperature of between 850-950° for approximately one hour with a ten minute anneal). Naturally, many techniques may be used to form a controlled thickness sacrificial base layer. For example, a thermally evaporated tungsten film can be used as a sacrificial base layer for polymer membranes and selectively removed with hydrogen peroxide. The basic requirement of the sacrificial base layer 28 is the ability to control the thickness with high precision across the entire wafer. Thermal oxidation of both polysilicon and nitride allows the control of the sacrificial layer thickness of less than 5% across the entire wafer. Limitations on this control arise from local inhomogeneities in the base layer, such as the initial thickness of the native oxide (especially for polysilicon), the grain size or density, and the impurity concentrations.

To mechanically connect the base layer 24 with the plug layer, which is necessary to maintain the pore spacing between layers, anchor points were defined in the sacrificial base layer 28. In the present design, this is accomplished by using the same mask shifted from the pore holes by 1 μm diagonally. This produced anchors in one or two corners of each pore hole, which provided the desired mechanical connection between the structural layers while opening as much pore area as possible. Figure 5 illustrates anchors 30 formed via this process.

A plug structural layer is subsequently deposited to file in the holes 26. This step has been implemented by depositing 1.5 μm of polysilicon. The resultant plug layer 32 is shown in Figure 6.

To open the pores at the surface, the plug layer 32 is planarzied down to the base layer, leaving the final structure with the plug layer only in the pore hole openings, as shown in Figure 7.

The method of planarization depends on the material used as the plug material. For the hard micro-fabrication materials (polysilicon and nitride), chemical mechanical polishing was used for planarization. The other materials studied were roughly planarized using a plasma etch, with a quick wet chemical smoothing. This technique has the advantage that, assuming it is not etched by the plasma used, the base layer is not affected, but has the disadvantage of the need for controlled etch timing to avoid completely etching the plugs themselves.

At this point, the membrane is ready for release, so a protective layer is deposited on the wafer (completely covering both sides of the wafer). Figure 8 illustrates a protective layer(s) 34.

The requirements of the protective layer 34 are that it be impervious to the silicon etch (KOH for these studies) and that it be removed without removing the plug 32 or base 24 structural layers. For polysilicon and nitride structural layers, a thin nitride layer is used as the protective layer (nitride is not etched at all by KOH and dissolves slowly in HF). For polymeric structural materials, silicone is used as a protective layer, due to the processing temperature necessary for nitride deposition. (835° C).

The backside etch windows were etched in the protective layer, exposing the silicon in desired areas. Then, the entire structure was placed in an 80° C KOH bath until the silicon wafer substrate 20 is etched up to the membrane base layer 24 (as evidenced by the smooth buried etch stop layer). Figure 8 illustrates the resultant aperture 36 formed in the substrate 20.

At this point, the buried sacrificial etch stop layer 22, the sacrificial oxide layer 34, and plug layer 32 are removed by etching in HF or SF_6/oxygen plasma. The resultant membrane 40 with nanometer scale pores is shown in Figure 9. Each hole 26 defines a nanometer scale pore, with the sacrificial base layer 28 providing aperture size control.

Figure 10 summarizes the foregoing processing steps. Figure 10 illustrates that the first processing step is to form a buried sacrificial etch stop layer on a substrate (step 50). A base layer is then constructed on the etch stop layer (step 52). Micrometer scale pores are then etched through the base layer to the etch stop layer (step 54). A sacrificial base layer is then deposited on the base layer (step 56).

Anchors are then patterned in the sacrificial base layer (step 58). A plug layer is then formed on the base layer (step 60). The plug layer is subsequently planarized (step 62) and polished (step 64). Protective layers are then formed on the base layer and substrate (step 66). The protective layers are then selectively etched to form an aperture in the substrate (step 68). The protective layer, plug layer, and portions of the buried sacrificial etch stop layer are then released (step 70) in the manner described above. Observe in Figure 9 that the buried sacrificial etch stop layer 22 is removed at the location of the pores, but remains between the base layer 24 and the substrate 20.

The performance of the membrane 40 of the invention was analyzed in comparison with two other types of membranes. In particular, a membrane 40 (with 24.5 nanometer pore size +/- 0.9 nm) of the invention was compared with porous alumina (i.e., a WHATMAN ANODISC membrane with a pore size of .02 microns) and a mixed celluose acetate and nitrate membrane (i.e., a MILLIPORE ISOPORE with a pore size of 0.025 microns). All membranes were examined *in vitro* by measuring relative concentrations of glucose on both sides of the microfabricated interface over time, using a mini diffusion chamber constructed around the membranes, as shown in Figure 11.

Figure 11 illustrates a chamber 80 with a first compartment 82 and a second compartment 84 with fixed volumes of 2 ml. Sampling ports 86 are provided in each compartment. The compartments are at least partially separated by the desired membrane 90. Preferably, the two compartments are sealed with o-rings and are screwed together.

Glucose is measured on either side of the membrane 90 using the diffusion chamber by means of a quantitative enzymatic assay (e.g., TRINDER, SIGMA) and colorometric reading via a spectrophotometer. Samples of 0.1 ml were taken from the diffusion chamber and 10 ul of that were added to 3 ml of glucose reagent in a cuvette, and were mixed gently by inversion. Each tube was incubated for 18 minutes at room temperature and then readings were taken at a wavelength of 505 nm. The reagent is linear up to 750 mg/dl. The diffusion chamber itself was attached to a motor for stirring in order to minimize boundary layer effects (diffusion resistance at the liquid/membrane interface). In order to ensure wetting of the pores, the receptor cell was first filled with phosphate buffer saline for fifteen minutes before the filling of the

donor cell. The donor cell was filled with solutions of glucose in phosphate buffer saline in varying concentrations. These tests were carried out at 37°C.

Albumin was measured on either side of the membrane using the same diffusion chamber. Albumin diffusion and/or exclusion was measured and quantified using Albumin BCP (bromocresol purple, Sigma.) A sample of 0.1 ml was taken at time zero and at the end of the diffusion period (time = 330 minutes). An aliquot of 300 ul was then added to 3 ml of the reagent and absorbance was read at 600 nm. Reagent plus deionized water was used as the blank. The assay is linear up to 6g/dl but is not accurate below 1 g/dl.

Figures 12-15 illustrate the results of these tests. The results illustrate that glucose concentration increases and begins to plateau at 330 minutes. Figure 12 shows the diffusion of glucose from a pure glucose solution and a mixed solution of glucose and albumin through 24.5 nm pore-sized silicon membranes.

The presence of albumin does not seem to impede passage of glucose through the membranes, nor slow down glucose transport in the experimental conditions employed. Figure 13 shows that no detectable amounts of albumin diffuse through the microfabricated membrane. The same membrane, however, shows glucose diffusion. The microfabricated membranes are able to achieve complete exclusion of albumin (to within the limits of detection), while allowing glucose diffusion.

Comparing these diffusion rates with those of commercially available membranes, it is seen in Figure 14 that microfabricated filters of the invention have glucose diffusion properties comparable to the MILLIPORE and WHITMAN membranes with similar pore size. However, when albumin diffusion is measured for all three membranes, the nanopore micromachined membranes of the invention have the greatest albumin exclusion, as shown in the table of Figure 16.

The foregoing results illustrate glucose diffusion test results of at least 1 mg/dl in 330 minutes. The membrane has an albumin diffusion test result of at most 0.1 g/dl in 330 minutes.

All of the membranes were evaluated before and after diffusion experiments to determine if any structural or surface changes had occurred. There were significant changes in membrane morphology for both the WHATMAN and MILLIPORE membranes after being incubated with glucose, albumin, and phosphate buffered saline

for over 24 hours at 37°C. In contrast, the micromachined silicon membrane has the same appearance before and after the tests. In fact, the microfabricated membrane pores are free from biofouling and any agglomeration of the protein in the pores. The MILLIPORE and WHATMAN membranes display inhomogeneities and morphological changes after all diffusion tests.

In sum, the microfabricated silicon membranes were characterized in terms of glucose diffusion, albumin exclusion and stability in biological environments. Results indicated that glucose does indeed diffuse through microfabricated membranes at a rate comparable to commercially available membranes. At the same time, albumin is excluded from passage. In a mixed solution of glucose and albumin, it has been shown that only glucose diffuses through the membranes. Although several membranes, such as those by WHATMAN and MILLIPORE are available for absolute filtration, these membranes do not have all the desired "ideal" membrane properties, such as stability, bio-compatibility, and well-controlled perm-selectivity.

The filter technology of the invention alleviates several of the problems associated with current commercially available separation membranes. Through the use of controlled sacrificial layer deposition, membranes can be fabricated with sufficient precision to guarantee high pore uniformity in sub-micron dimensions. The thickness of the thermally grown oxide can be controlled to +/- 1nm for nominal pore sizes as small as 18nm. This is the size range needed to obtain absolute protein exclusion and glucose diffusion for biosensor applications. Moreover, this filter technology can bring in the added advantages of stability, minimal protein adsorption through established silicon surface modification techniques, reusability, and sterilizability.

The invention has been disclosed in connection with fabricated elemental silicon. The techniques of the invention may also be used in connection with other bio-compatible materials, such as metals (e.g., titanium), ceramics (e.g., silica or silicon nitride), and polymers (e.g., polytetrafluorethylene, polymethylmethacrylate, polystyrenes, and silicones).

The foregoing description, for purposes of explanation, used specific nomenclature to provide a thorough understanding of the invention. However, it will be apparent to one skilled in the art that the specific details are not required in order to

practice the invention. In other instances, well known circuits and devices are shown in block diagram form in order to avoid unnecessary distraction from the underlying invention. Thus, the foregoing descriptions of specific embodiments of the present invention are presented for purposes of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed, obviously many modifications and variations are possible in view of the above teachings. The embodiments were chosen and described in order to best explain the principles of the invention and its practical applications, to thereby enable others skilled in the art to best utilize the invention and various embodiments with various modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the following claims and their equivalents.

IN THE CLAIMS:

1. A method of forming a membrane with nanometer scale pores, said method comprising the steps of:

forming a sacrificial etch stop layer on a substrate;

constructing a base layer on said sacrificial etch stop layer;

building a sacrificial base layer on said base layer; and

removing said sacrificial base layer from selected regions of said base layer to define nanometer scale pores within said base layer.

2. The method of claim 1 wherein said constructing a base layer includes constructing a base layer with micrometer scale pores, said micrometer scale pores being constricted to said nanometer scale pores after sacrificial base layer is removed from selected regions of said base layer.

3. The method of claim 1 further comprising the step of patterning anchors in said sacrificial base layer.

4. The method of claim 3 further comprising the step of forming a plug layer on said base layer.

5. The method of claim 4 further comprising the step of planarizing said plug layer.

6. The method of claim 5 wherein said planarizing step includes the step of chemically mechanically polishing said plug layer.

7. The method of claim 5 further comprising the step of forming a protective layer on said base layer and said substrate.

8. The method of claim 7 further comprising the step of selectively etching said protective layer to form an aperture in said substrate.

9. The method of claim 8 further comprising the step of releasing said protective layer.

10. The method of claim 8 further comprising the step of releasing said plug layer.

11. The method of claim 8 further comprising the step of selectively releasing said sacrificial etch stop layer.

12. The method of claim 1 wherein forming said sacrificial etch stop layer on said substrate includes forming a silicon nitride layer on said substrate.

13. An apparatus formed by the method of claim 1.

14. A filter, comprising:
a membrane of elemental silicon with sub-fifty nanometer pores formed therein.

15. The filter of claim 14 wherein said membrane has a glucose diffusion test result of at least 1 mg/dl and an albumin diffusion test result of at most 0.1 g/dl.

16. The filter of claim 14 wherein said glucose diffusion test and said albumin diffusion test are performed over 330 minutes.

17. The filter of claim 14 further comprising:
a substrate;
a buried sacrificial etch stop layer positioned on said substrate; and
said membrane positioned on said buried sacrificial etch stop layer.

18. The filter of claim 17 wherein said buried sacrificial etch stop layer is silicon nitride.

19. The filter of claim 17 further comprising oxide anchors formed between said membrane and said buried sacrificial etch stop layer.

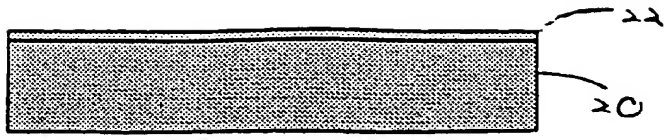


Fig. 1

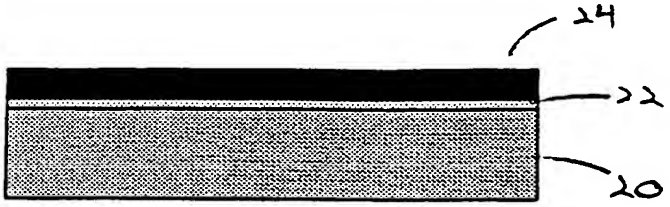


Fig. 2

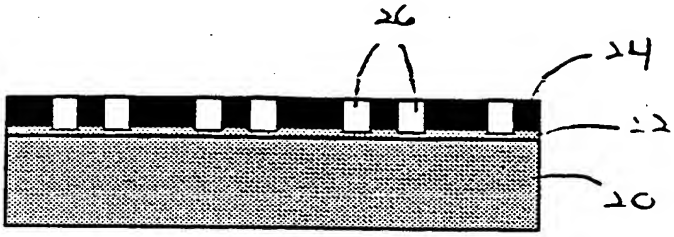


Fig. 3

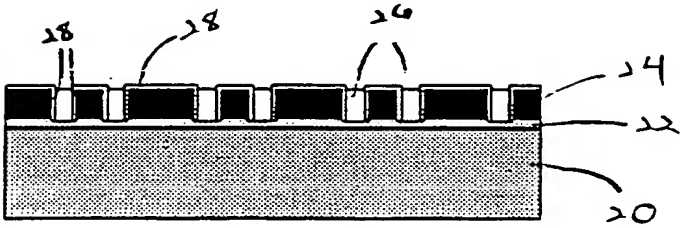


Fig. 4

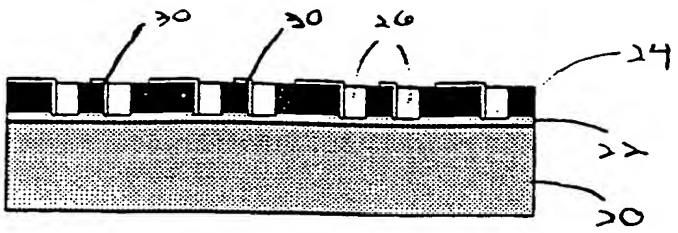


Fig. 5

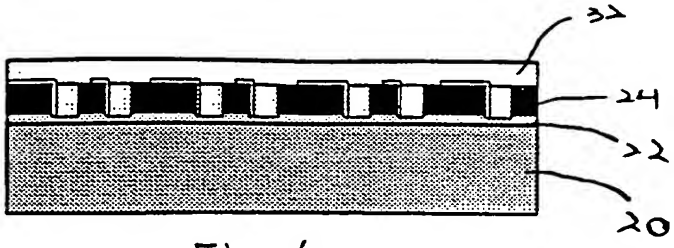


Fig. 6

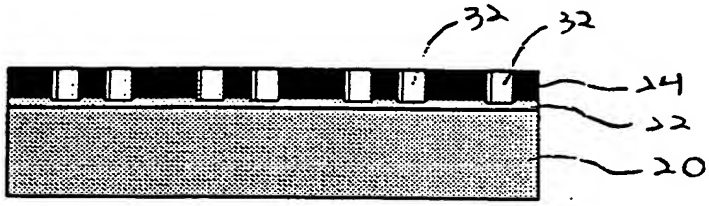


Fig. 7

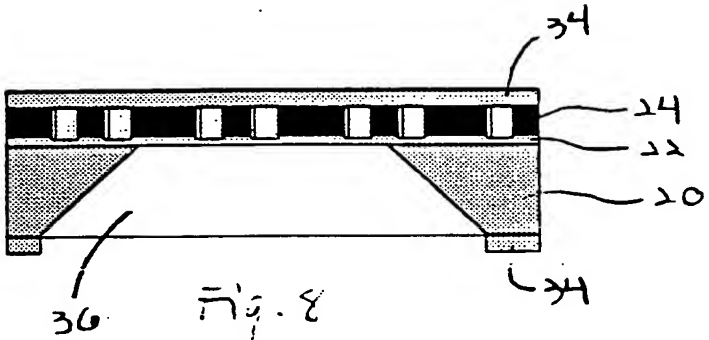


Fig. 8

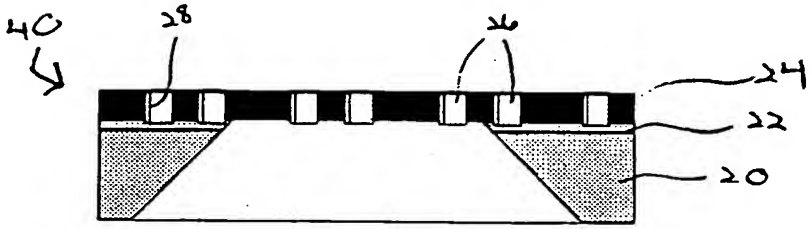


Fig. 9

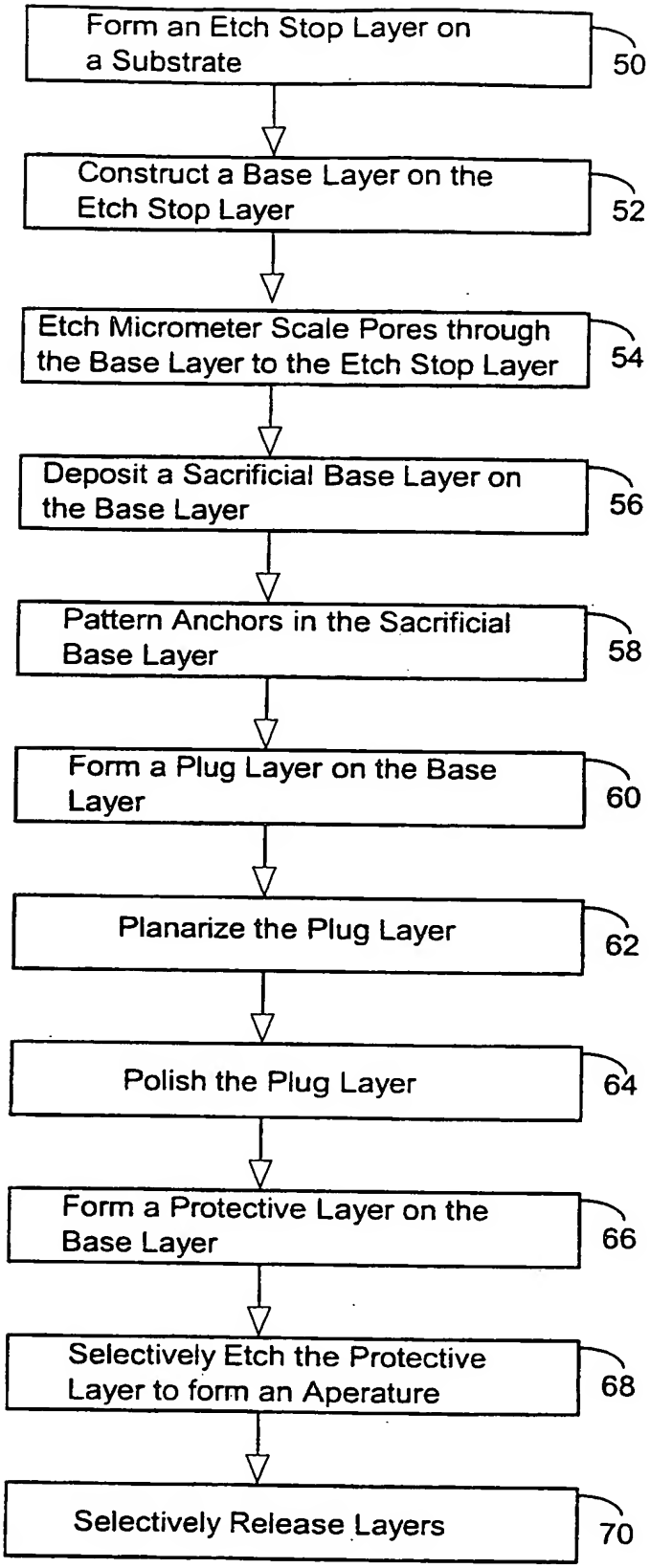


Figure 10

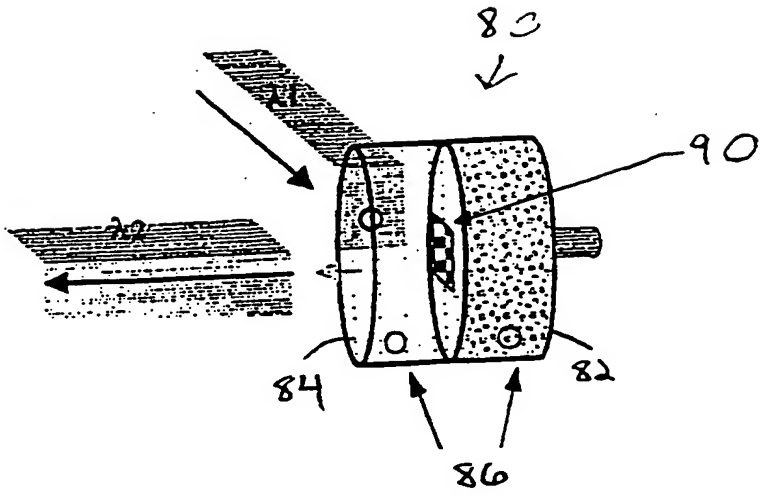


Fig. 11

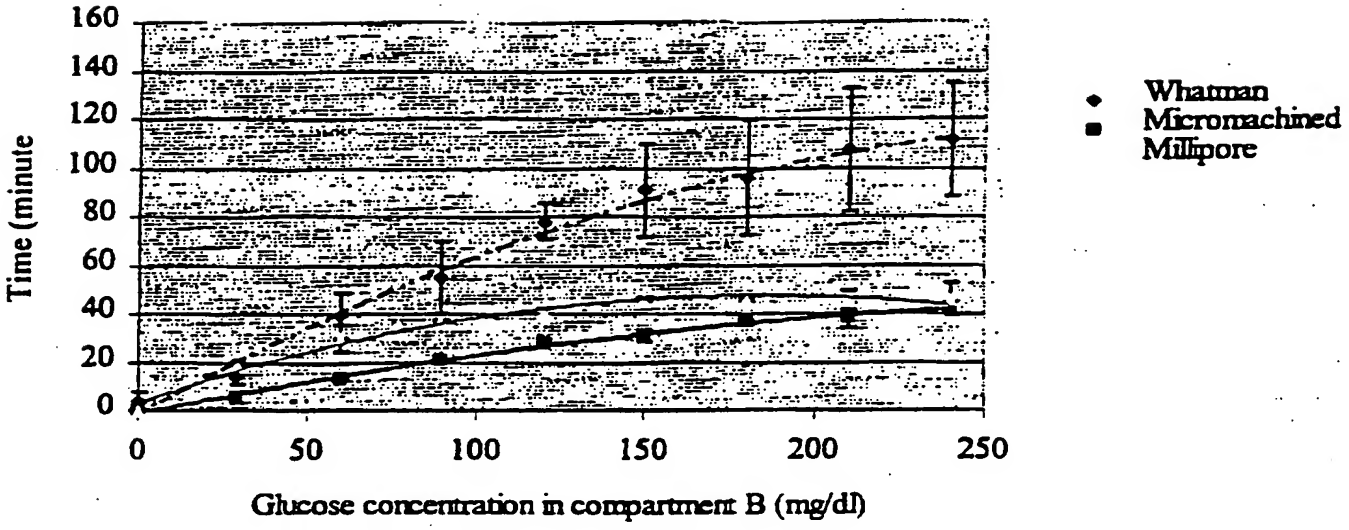


Fig. 12

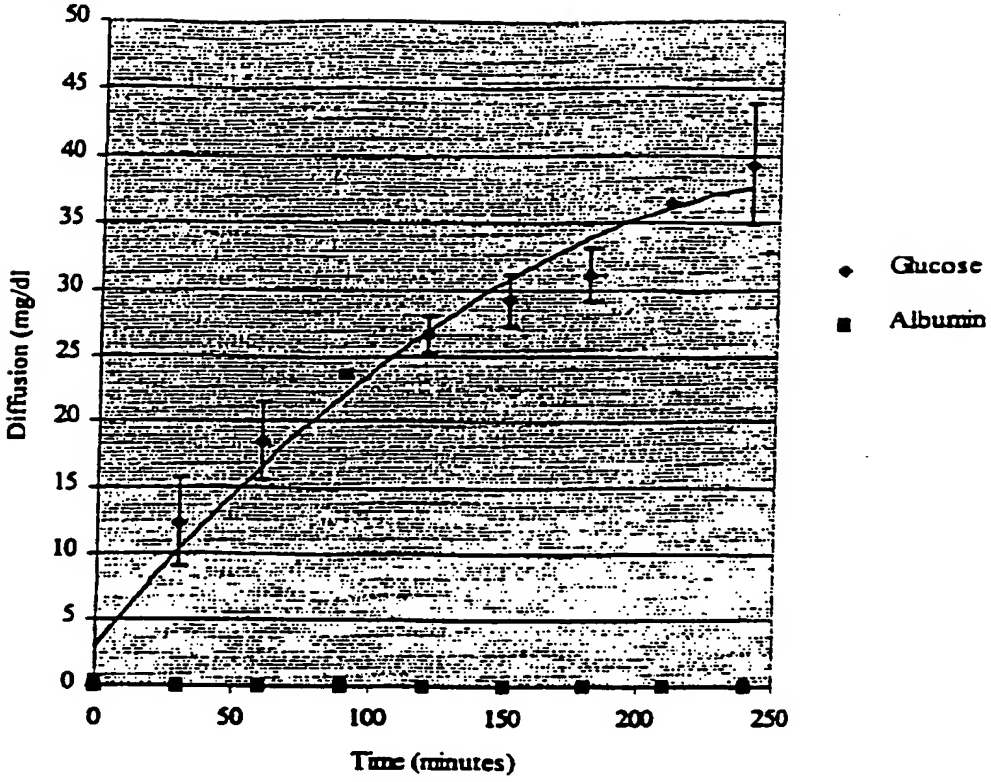


Fig. 13

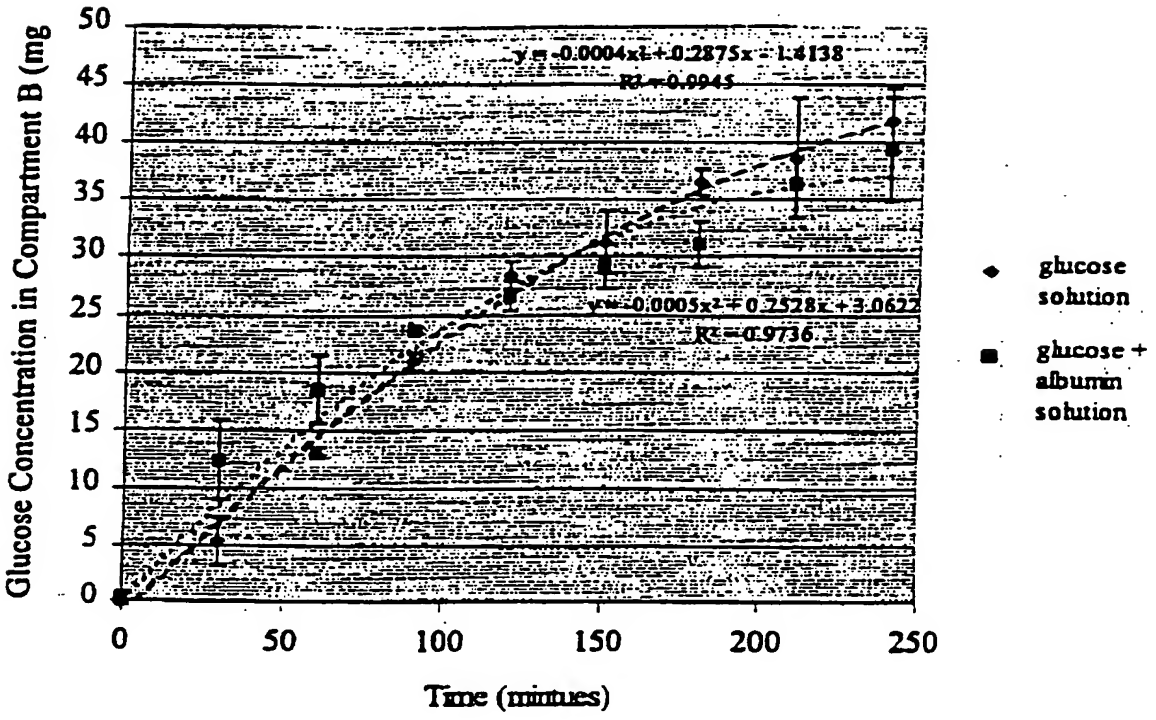


Fig. 14

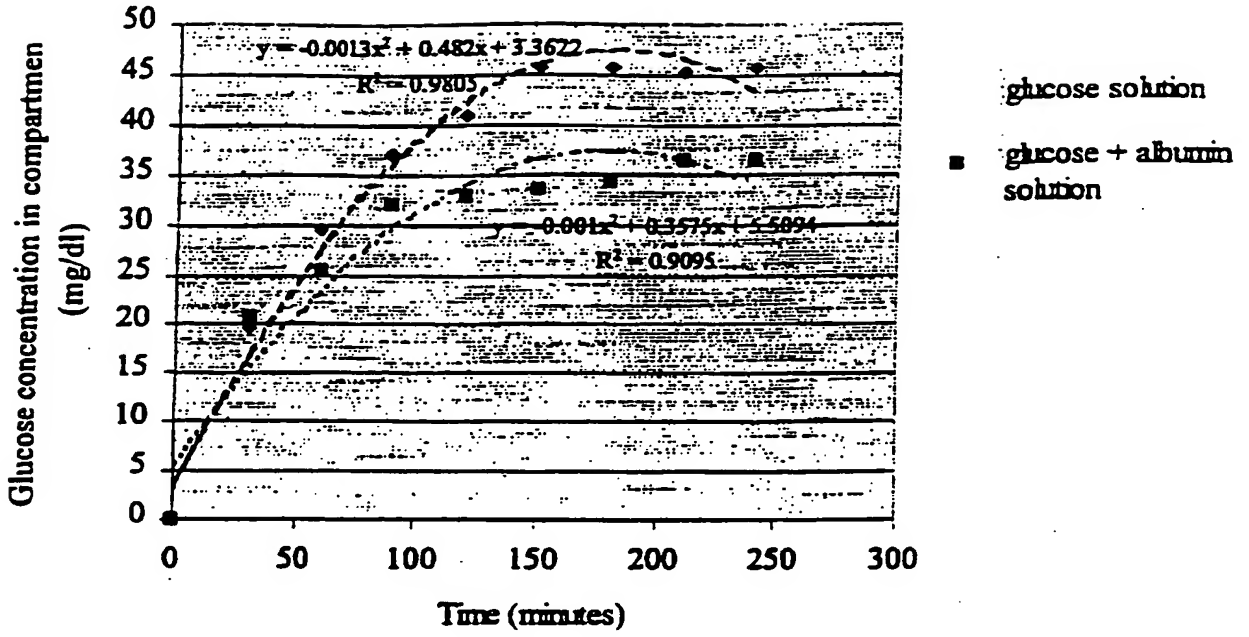


Fig. 15

	WHATMAN		MILLIPORE		MICROMACHINED	
TIME	ALBUMIN CONC.		ALBUMIN CONC.		ALBUMIN CONC.	
minutes	ABS.	g/dL	g/dL	g/dL	ABS.	g/dL
0	0.381	3.88	0.423	4.31	0.395	3.980
420	0.352	3.58	0.398	4.05	0.394	3.970
Amount of Albumin diffused		0.3 g/dL		0.26 g/dL		0.01 g/dL

Fig. 16

INTERNATIONAL SEARCH REPORT

Intern: ial application No.
PCT/US00/31749

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : B81C 1/00; B82B 1/00, 3/00; B01D 63/00
US CL : 216/2, 56, 88, 89; 10/321.84, 323.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 216/2, 56, 88, 89; 10/321.84, 323.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
none

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,919,364 A (LEBOUITZ et al) 06 July 1999 (06.07.1999), Fig.4-11, column 4, line 60-column 6, line 10.	1-19
Y	US 5,948,255 A (KELLER et al) 07 September 1999 (07.09.1999), Fig.1A-1G, column 3, line 66-column 5, line 20.	1-4, 13-19
Y	US 5,770,076 A (CHU et al) 23 June 1998 (23.06.1998), columns 4-8.	1-19
Y	US 5,798,042 A (CHU et al) 25 August 1998 (25.08.1998), abstract	1-19
Y	US 5,938,923 A (TU et al) 17 August 1999 (17.08.1999), abstract.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2001 (28.01.2001)	21 MAR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Anita Alanko Telephone No. 703-308-0661

Form PCT/ISA/210 (second sheet) (July 1998)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 May 2001 (25.05.2001)

PCT

(10) International Publication Number
WO 01/036321 A1

(51) International Patent Classification[7]: B81C 1/00, B82B 1/00, 3/00, B01D 63/00

(21) International Application Number: PCT/US00/31749

(22) International Filing Date:
17 November 2000 (17.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/166,049 17 November 1999 (17.11.1999) US

(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, Fifth floor, Oakland, CA 94607-5200 (US).

(72) Inventors: **HANSFORD, Derek**; 2719 Quarry Vally Road, Columbus, OH 43204 (US). **FERRARI, Mauro**; 8189 Crossgate North Court, Dublin, OH 43017 (US).

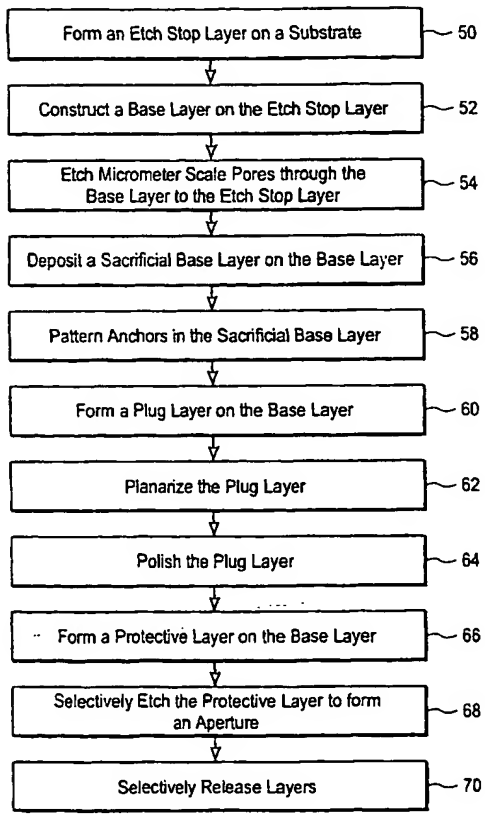
(74) Agents: **GALLIANI, William, S.** et al.; Pennie & Edmonds LLP, 1155 Avenue of the Americas, New York, NY 10036 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian

[Continued on next page]

(54) Title: APPARATUS AND METHOD FOR FORMING A MEMBRANE WITH NANOMETER SCALE PORES



(57) Abstract: A method of forming a membrane with nanometer scale pores includes forming a sacrificial etch stop layer on a substrate. A base layer is constructed on the sacrificial etch stop layer. Micrometer scale pores are formed within the base layer. A sacrificial base layer is built on the base layer. The sacrificial base layer is removed from selected regions of the base layer to define nanometer scale pores within the base layer. The resultant membrane has sub-fifty nanometer pores formed within it.

WO 01/036321 A1

patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(48) Date of publication of this corrected version:
4 July 2002

(15) Information about Correction:
see PCT Gazette No. 27/2002 of 4 July 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

APPARATUS AND METHOD FOR FORMING A MEMBRANE WITH NANOMETER SCALE PORES

This application claims priority to the U.S. Provisional Patent Application entitled, "Apparatus and Method for Forming a Membrane with Nanometer Scale Pores," Serial Number 60/166,049, filed November 17, 1999.

BRIEF DESCRIPTION OF THE INVENTION

This invention relates generally to membranes with nanometer scale pores that may be used in filtering applications. More particularly, this invention relates to the use of microfabrication processing techniques to form nanometer scale porous membranes.

BACKGROUND OF THE INVENTION

There is a revolution occurring in biological research. Emphasis is rapidly shifting towards the view of biology in terms of a complex series of physical and chemical interactions, and interdisciplinary research between engineers, biologists, physicists, and clinicians is becoming prevalent. A rapidly developing field of research is the use of micro-fabrication to make mechanically, electrically, and/or chemically interactive structures for biological research and applications, known collectively as BioMEMS, with the term "Bio" referring to biology, and the term "MEMS" referring to MicroElectroMechanical devices. By using semiconductor based micro-fabrication techniques, MEM structures can be fabricated with spatial features from the sub-micron range up to several millimeters. These multi-scale structures correspond well with hierarchical biological structures, from proteins and sub-cellular organelles to the tissue and organ levels. This structural correlation allows scientists to investigate biological structure on their respective size scales and

interact in more appropriate and responsive manners to the structures within the body and within biological fluids.

Conceivably, it would be desirable to use standard micro-lithography to produce structures that can be used for basic biological research, diagnostic, and therapeutic applications. However, conventional lithographic techniques have feature size limitations that prevent their use for fabricating structures that can physically interact with molecules of biological interest, such as proteins, nucleotides, and various physiological nutrients. To interact directly with these molecules, features must be fabricated with sizes less than 50 nm, which is not projected for state of the art lithography until the year 2008. Furthermore, because of the fabrication techniques which are used for MEMS structures and the potential for contamination they introduce, state of the art equipment will not be used to fabricate these structures, leading to a further delay in the fabrication of direct interaction structures.

In view of the foregoing, it would be highly desirable to provide a technique for fabricating membranes with features less than 50 nanometers. Ideally, such a technique would rely upon standard lithography processing techniques and would yield a device that is compatible with biological research, diagnostic, and therapeutic applications.

SUMMARY OF THE INVENTION

The invention includes a filter comprising a membrane of elemental silicon with sub-fifty nanometer pores formed within it. The membrane has a glucose diffusion test result of at least 1 mg/dl and an albumin diffusion test result of at most 0.1 g/dl. The filter has a substrate, a buried sacrificial etch stop layer positioned on the substrate, with the membrane positioned on the buried sacrificial etch stop layer. In one embodiment, the buried sacrificial etch stop layer is silicon nitride.

The method of the invention includes forming a membrane with nanometer scale pores. A sacrificial etch stop layer is formed on a substrate. A base layer is constructed on the sacrificial etch stop layer. Micrometer scale pores are formed within the base layer. A sacrificial base layer is built on the base layer. The sacrificial base layer is removed from selected regions of the base layer to define nanometer scale pores within the base layer.

BRIEF DESCRIPTION OF THE DRAWINGS

For a better understanding of the invention, reference should be made to the following detailed description taken in conjunction with the accompanying drawings, in which:

FIGURE 1 illustrates a substrate with a sacrificial buried layer formed thereon in accordance with an embodiment of the invention.

FIGURE 2 illustrates a base layer formed on the sacrificial buried layer of Figure 1.

FIGURE 3 illustrates etched micrometer pores formed within a base layer and stopped by a sacrificial buried layer in accordance with the invention.

FIGURE 4 illustrates the deposition of a sacrificial base layer in accordance with an embodiment of the invention.

FIGURE 5 illustrates anchors formed in the sacrificial base layer utilized in accordance with the invention.

FIGURE 6 illustrates a plug layer formed in accordance with an embodiment of the invention.

FIGURE 7 illustrates the plug layer after mechanical polishing in accordance with an embodiment of the invention.

FIGURE 8 illustrates a protective layer and resultant selective etching utilized in accordance with an embodiment of the invention.

FIGURE 9 illustrates a fully released nanometer scale membrane after removal of the protective layers, and selective regions of the sacrificial buried layer.

FIGURE 10 illustrates processing steps used to construct the devices of Figures 1-9.

FIGURE 11 illustrates a device used to test the membrane of the invention.

FIGURE 12 illustrates glucose diffusion through three different nanopore membranes.

FIGURE 13 illustrates diffusion of glucose and albumin through micromachined nanopore membranes.

FIGURE 14 illustrates glucose diffusion through micromachined membranes incubated in pure glucose and mixed glucose/albumin solutions.

FIGURE 15 illustrates diffusion through millipore membranes incubated in pure glucose and mixed glucose/albumin solutions.

FIGURE 16 is a table illustrating diffusion of Albumin through various membranes.

Like reference numerals refer to corresponding parts throughout the drawings.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relies upon many prior art techniques in forming a membrane with nanometer scale pores. However, the invention also departs from the prior art in several key respects. These departures from the prior art facilitate the formation of pores less than 50 nanometer.

First, the technique of the invention relies upon a buried sacrificial etch stop layer. By way of the example, the buried sacrificial etch stop layer may be silicon nitride. The buried sacrificial etch stop layer operates as an etch stop, and is then removed to expose the nanopores of the invention.

The invention's use of a buried sacrificial etch stop layer as an etchant stop during the formation of nanometer scale pores is believed to be novel. While buried etch stop layers are used for structural purposes in the prior art, it is not believed that the prior art shows or suggests the formation of a buried etch stop layer, which operates as an etchant stop during the formation of pores, and which is subsequently etched away to expose pores.

The buried sacrificial etch stop layer facilitates three-dimensional control of the pore structure. Prior art techniques endeavored to control pore structure by balancing the etching of two different layers. The buried sacrificial etch stop technique of the invention facilitates the formation of pores less than 50 nanometers. Moreover, these pores can be uniformly formed across the entire wafer.

The buried sacrificial etch stop layer of the invention eliminates the prior art use of diffused boron. When diffused boron is used as an etch stop it provides an imprecise membrane depth. In addition, boron introduces stresses into the completed membrane.

Advantageously, the buried sacrificial etch stop layer of the invention provides absolute etching selectivity, as the layer will not be etched at all by the disclosed KOH etchant. In contrast, boron will be minimally etched in the presence of a KOH etchant.

The technique of the invention departs from prior art techniques in another important manner. Namely, the technique of the invention relies upon planarization of the outer structural layer to expose the total pore area, instead of the prior art approach of etching entrance holes in the top layer.

Preferably, the first step in the fabrication protocol is to etch a support ridge structure into a substrate. This is accomplished by simply etching a ridge structure prior to the deposition of the etch stop layer. The ridge provides mechanical rigidity to the subsequently formed membrane structure.

The buried sacrificial etch stop layer is then deposited on the substrate. For example, a low stress silicon nitride (LSN or nitride) is deposited on the substrate using low pressure chemical vapor depositions (LPCVD). In one embodiment, 0.4 μm of silicon nitride was used. The resultant structure is shown in Figure 1. Figure 1 illustrates a substrate 20 with a sacrificial etch stop layer 22 formed thereon.

The base structural layer (base layer) of the membrane is deposited on top of the stop layer 22. Because the stop layer 22 is thin, the structural layer is deposited down into the support ridges formed in the substrate 20. In one embodiment, 5 μm of polysilicon is used as the base layer. Figure 2 illustrates the base layer 24 positioned on the stop layer 22. Low stress silicon nitride may also be used as the base layer, in which case it operates as its own etch stop layer.

The next processing step is to etch holes in the base layer 24 to define the shape of the pores. Prior art masks may be used to define the pores. For example, the holes may be etched through the polysilicon by chlorine plasma, with a thermally grown oxide layer used as a mask. In this step, it is important to make sure the etching goes completely through the base layer 24, so a 10-15% overetch is preferably used. It is useful to note that the buried sacrificial etch stop layer 22 acts as an etch stop for the plasma etching of a silicon base layer 24. Otherwise, if the plasma punched through the etch stop layer, tighter control of the etch step layer would have to be exercised to prevent the complete removal of the nitride under the plug layer (to prevent removal in the final KOH etch). Figure 3 illustrates the result of this processing. In particular, the

figure illustrates holes 26 formed in the base layer 24, but terminating in the buried sacrificial etch stop layer 22. At this stage, the holes 26 define micrometer scale pores.

Pore sacrificial oxide is subsequently grown on the base layer 24. Figure 4 illustrates a sacrificial oxide 28 positioned on the base layer 24. This sacrificial oxide 28 is also referred to as a nanometer scale sacrificial base layer or sacrificial base layer. This sacrificial base layer 28 is used to define nanometer scale pores.

The thickness of the sacrificial base layer 28 determined the pore size in the final membrane, so control of this step is critical to reproducible membranes. This is accomplished by the thermal oxidation of the base layer 24 (e.g., a growth temperature of between 850-950° for approximately one hour with a ten minute anneal). Naturally, many techniques may be used to form a controlled thickness sacrificial base layer. For example, a thermally evaporated tungsten film can be used as a sacrificial base layer for polymer membranes and selectively removed with hydrogen peroxide. The basic requirement of the sacrificial base layer 28 is the ability to control the thickness with high precision across the entire wafer. Thermal oxidation of both polysilicon and nitride allows the control of the sacrificial layer thickness of less than 5% across the entire wafer. Limitations on this control arise from local inhomogeneities in the base layer, such as the initial thickness of the native oxide (especially for polysilicon), the grain size or density, and the impurity concentrations.

To mechanically connect the base layer 24 with the plug layer, which is necessary to maintain the pore spacing between layers, anchor points were defined in the sacrificial base layer 28. In the present design, this is accomplished by using the same mask shifted from the pore holes by 1 μm diagonally. This produced anchors in one or two corners of each pore hole, which provided the desired mechanical connection between the structural layers while opening as much pore area as possible. Figure 5 illustrates anchors 30 formed via this process.

A plug structural layer is subsequently deposited to file in the holes 26. This step has been implemented by depositing 1.5 μm of polysilicon. The resultant plug layer 32 is shown in Figure 6.

To open the pores at the surface, the plug layer 32 is planarzied down to the base layer, leaving the final structure with the plug layer only in the pore hole openings, as shown in Figure 7.

The method of planarization depends on the material used as the plug material. For the hard micro-fabrication materials (polysilicon and nitride), chemical mechanical polishing was used for planarization. The other materials studied were roughly planarized using a plasma etch, with a quick wet chemical smoothing. This technique has the advantage that, assuming it is not etched by the plasma used, the base layer is not affected, but has the disadvantage of the need for controlled etch timing to avoid completely etching the plugs themselves.

At this point, the membrane is ready for release, so a protective layer is deposited on the wafer (completely covering both sides of the wafer). Figure 8 illustrates a protective layer(s) 34.

The requirements of the protective layer 34 are that it be impervious to the silicon etch (KOH for these studies) and that it be removed without removing the plug 32 or base 24 structural layers. For polysilicon and nitride structural layers, a thin nitride layer is used as the protective layer (nitride is not etched at all by KOH and dissolves slowly in HF). For polymeric structural materials, silicone is used as a protective layer, due to the processing temperature necessary for nitride deposition. (835° C).

The backside etch windows were etched in the protective layer, exposing the silicon in desired areas. Then, the entire structure was placed in an 80° C KOH bath until the silicon wafer substrate 20 is etched up to the membrane base layer 24 (as evidenced by the smooth buried etch stop layer). Figure 8 illustrates the resultant aperture 36 formed in the substrate 20.

At this point, the buried sacrificial etch stop layer 22, the sacrificial oxide layer 34, and plug layer 32 are removed by etching in HF or SF_6/oxygen plasma. The resultant membrane 40 with nanometer scale pores is shown in Figure 9. Each hole 26 defines a nanometer scale pore, with the sacrificial base layer 28 providing aperture size control.

Figure 10 summarizes the foregoing processing steps. Figure 10 illustrates that the first processing step is to form a buried sacrificial etch stop layer on a substrate (step 50). A base layer is then constructed on the etch stop layer (step 52). Micrometer scale pores are then etched through the base layer to the etch stop layer (step 54). A sacrificial base layer is then deposited on the base layer (step 56).

Anchors are then patterned in the sacrificial base layer (step 58). A plug layer is then formed on the base layer (step 60). The plug layer is subsequently planarized (step 62) and polished (step 64). Protective layers are then formed on the base layer and substrate (step 66). The protective layers are then selectively etched to form an aperture in the substrate (step 68). The protective layer, plug layer, and portions of the buried sacrificial etch stop layer are then released (step 70) in the manner described above. Observe in Figure 9 that the buried sacrificial etch stop layer 22 is removed at the location of the pores, but remains between the base layer 24 and the substrate 20.

The performance of the membrane 40 of the invention was analyzed in comparison with two other types of membranes. In particular, a membrane 40 (with 24.5 nanometer pore size +/- 0.9 nm) of the invention was compared with porous alumina (i.e., a WHATMAN ANODISC membrane with a pore size of .02 microns) and a mixed celluose acetate and nitrate membrane (i.e., a MILLIPORE ISOPORE with a pore size of 0.025 microns). All membranes were examined *in vitro* by measuring relative concentrations of glucose on both sides of the microfabricated interface over time, using a mini diffusion chamber constructed around the membranes, as shown in Figure 11.

Figure 11 illustrates a chamber 80 with a first compartment 82 and a second compartment 84 with fixed volumes of 2 ml. Sampling ports 86 are provided in each compartment. The compartments are at least partially separated by the desired membrane 90. Preferably, the two compartments are sealed with o-rings and are screwed together.

Glucose is measured on either side of the membrane 90 using the diffusion chamber by means of a quantitative enzymatic assay (e.g., TRINDER, SIGMA) and colorometric reading via a spectrophotometer. Samples of 0.1 ml were taken from the diffusion chamber and 10 ul of that were added to 3 ml of glucose reagent in a cuvette, and were mixed gently by inversion. Each tube was incubated for 18 minutes at room temperature and then readings were taken at a wavelength of 505 nm. The reagent is linear up to 750 mg/dl. The diffusion chamber itself was attached to a motor for stirring in order to minimize boundary layer effects (diffusion resistance at the liquid/membrane interface). In order to ensure wetting of the pores, the receptor cell was first filled with phosphate buffer saline for fifteen minutes before the filling of the

donor cell. The donor cell was filled with solutions of glucose in phosphate buffer saline in varying concentrations. These tests were carried out at 37°C.

Albumin was measured on either side of the membrane using the same diffusion chamber. Albumin diffusion and/or exclusion was measured and quantified using Albumin BCP (bromocresol purple, Sigma.) A sample of 0.1 ml was taken at time zero and at the end of the diffusion period (time = 330 minutes). An aliquot of 300 ul was then added to 3 ml of the reagent and absorbance was read at 600 nm. Reagent plus deionized water was used as the blank. The assay is linear up to 6g/dl but is not accurate below 1 g/dl.

Figures 12-15 illustrate the results of these tests. The results illustrate that glucose concentration increases and begins to plateau at 330 minutes. Figure 12 shows the diffusion of glucose from a pure glucose solution and a mixed solution of glucose and albumin through 24.5 nm pore-sized silicon membranes.

The presence of albumin does not seem to impede passage of glucose through the membranes, nor slow down glucose transport in the experimental conditions employed. Figure 13 shows that no detectable amounts of albumin diffuse through the microfabricated membrane. The same membrane, however, shows glucose diffusion. The microfabricated membranes are able to achieve complete exclusion of albumin (to within the limits of detection), while allowing glucose diffusion.

Comparing these diffusion rates with those of commercially available membranes, it is seen in Figure 14 that microfabricated filters of the invention have glucose diffusion properties comparable to the MILLIPORE and WHITMAN membranes with similar pore size. However, when albumin diffusion is measured for all three membranes, the nanopore micromachined membranes of the invention have the greatest albumin exclusion, as shown in the table of Figure 16.

The foregoing results illustrate glucose diffusion test results of at least 1 mg/dl in 330 minutes. The membrane has an albumin diffusion test result of at most 0.1 g/dl in 330 minutes.

All of the membranes were evaluated before and after diffusion experiments to determine if any structural or surface changes had occurred. There were significant changes in membrane morphology for both the WHATMAN and MILLIPORE membranes after being incubated with glucose, albumin, and phosphate buffered saline

for over 24 hours at 37°C. In contrast, the micromachined silicon membrane has the same appearance before and after the tests. In fact, the microfabricated membrane pores are free from biofouling and any agglomeration of the protein in the pores. The MILLIPORE and WHATMAN membranes display inhomogeneities and morphological changes after all diffusion tests.

In sum, the microfabricated silicon membranes were characterized in terms of glucose diffusion, albumin exclusion and stability in biological environments. Results indicated that glucose does indeed diffuse through microfabricated membranes at a rate comparable to commercially available membranes. At the same time, albumin is excluded from passage. In a mixed solution of glucose and albumin, it has been shown that only glucose diffuses through the membranes. Although several membranes, such as those by WHATMAN and MILLIPORE are available for absolute filtration, these membranes do not have all the desired "ideal" membrane properties, such as stability, bio-compatibility, and well-controlled perm-selectivity.

The filter technology of the invention alleviates several of the problems associated with current commercially available separation membranes. Through the use of controlled sacrificial layer deposition, membranes can be fabricated with sufficient precision to guarantee high pore uniformity in sub-micron dimensions. The thickness of the thermally grown oxide can be controlled to +/- 1nm for nominal pore sizes as small as 18nm. This is the size range needed to obtain absolute protein exclusion and glucose diffusion for biosensor applications. Moreover, this filter technology can bring in the added advantages of stability, minimal protein adsorption through established silicon surface modification techniques, reusability, and sterilizability.

The invention has been disclosed in connection with fabricated elemental silicon. The techniques of the invention may also be used in connection with other bio-compatible materials, such as metals (e.g., titanium), ceramics (e.g., silica or silicon nitride), and polymers (e.g., polytetrafluorethylene, polymethylmethacrylate, polystyrenes, and silicones).

The foregoing description, for purposes of explanation, used specific nomenclature to provide a thorough understanding of the invention. However, it will be apparent to one skilled in the art that the specific details are not required in order to

practice the invention. In other instances, well known circuits and devices are shown in block diagram form in order to avoid unnecessary distraction from the underlying invention. Thus, the foregoing descriptions of specific embodiments of the present invention are presented for purposes of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed, obviously many modifications and variations are possible in view of the above teachings. The embodiments were chosen and described in order to best explain the principles of the invention and its practical applications, to thereby enable others skilled in the art to best utilize the invention and various embodiments with various modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the following claims and their equivalents.

IN THE CLAIMS:

1. A method of forming a membrane with nanometer scale pores, said method comprising the steps of:

forming a sacrificial etch stop layer on a substrate;

constructing a base layer on said sacrificial etch stop layer;

building a sacrificial base layer on said base layer; and

removing said sacrificial base layer from selected regions of said base layer to define nanometer scale pores within said base layer.

2. The method of claim 1 wherein said constructing a base layer includes constructing a base layer with micrometer scale pores, said micrometer scale pores being constricted to said nanometer scale pores after sacrificial base layer is removed from selected regions of said base layer.

3. The method of claim 1 further comprising the step of patterning anchors in said sacrificial base layer.

4. The method of claim 3 further comprising the step of forming a plug layer on said base layer.

5. The method of claim 4 further comprising the step of planarizing said plug layer.

6. The method of claim 5 wherein said planarizing step includes the step of chemically mechanically polishing said plug layer.

7. The method of claim 5 further comprising the step of forming a protective layer on said base layer and said substrate.

8. The method of claim 7 further comprising the step of selectively etching said protective layer to form an aperture in said substrate.

9. The method of claim 8 further comprising the step of releasing said protective layer.

10. The method of claim 8 further comprising the step of releasing said plug layer.

11. The method of claim 8 further comprising the step of selectively releasing said sacrificial etch stop layer.

12. The method of claim 1 wherein forming said sacrificial etch stop layer on said substrate includes forming a silicon nitride layer on said substrate.

13. An apparatus formed by the method of claim 1.

14. A filter, comprising:
a membrane of elemental silicon with sub-fifty nanometer pores formed therein.

15. The filter of claim 14 wherein said membrane has a glucose diffusion test result of at least 1 mg/dl and an albumin diffusion test result of at most 0.1 g/dl.

16. The filter of claim 14 wherein said glucose diffusion test and said albumin diffusion test are performed over 330 minutes.

17. The filter of claim 14 further comprising:
a substrate;
a buried sacrificial etch stop layer positioned on said substrate; and
said membrane positioned on said buried sacrificial etch stop layer.

18. The filter of claim 17 wherein said buried sacrificial etch stop layer is silicon nitride.

19. The filter of claim 17 further comprising oxide anchors formed between said membrane and said buried sacrificial etch stop layer.

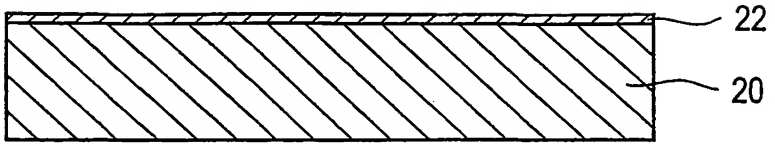


FIG. 1

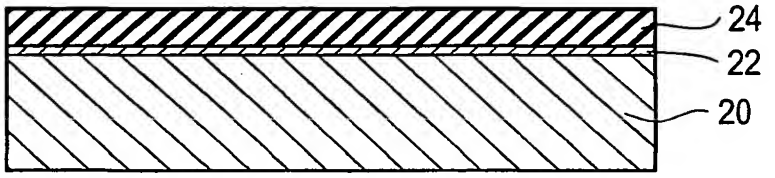


FIG. 2

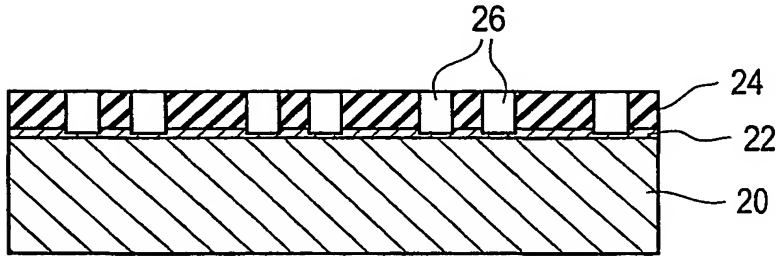


FIG. 3

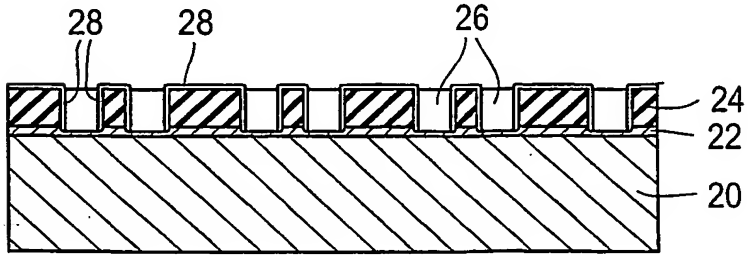


FIG. 4

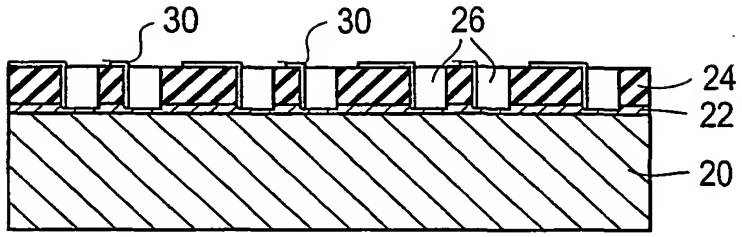


FIG. 5

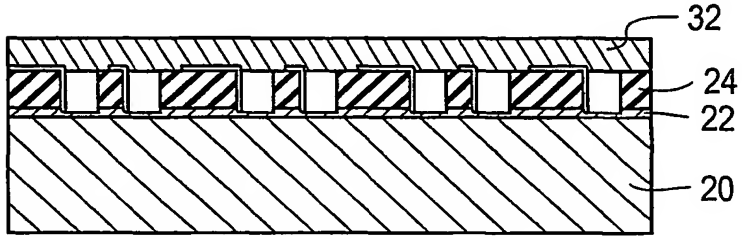


FIG. 6

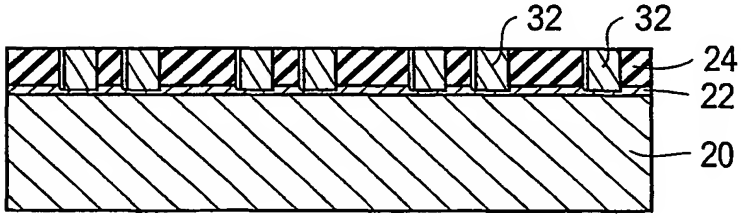


FIG.7

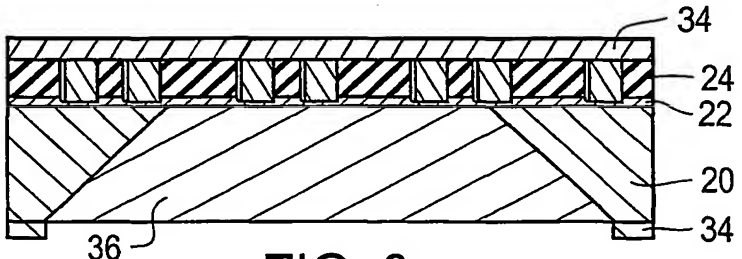


FIG. 8

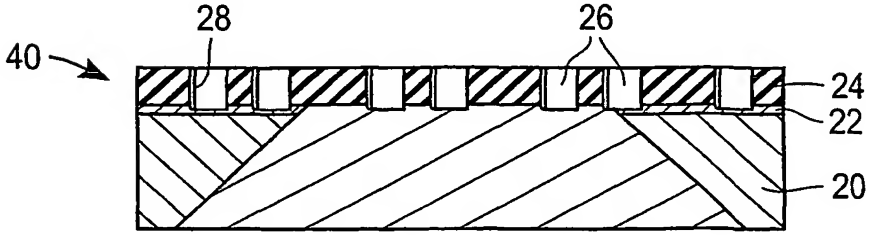


FIG. 9

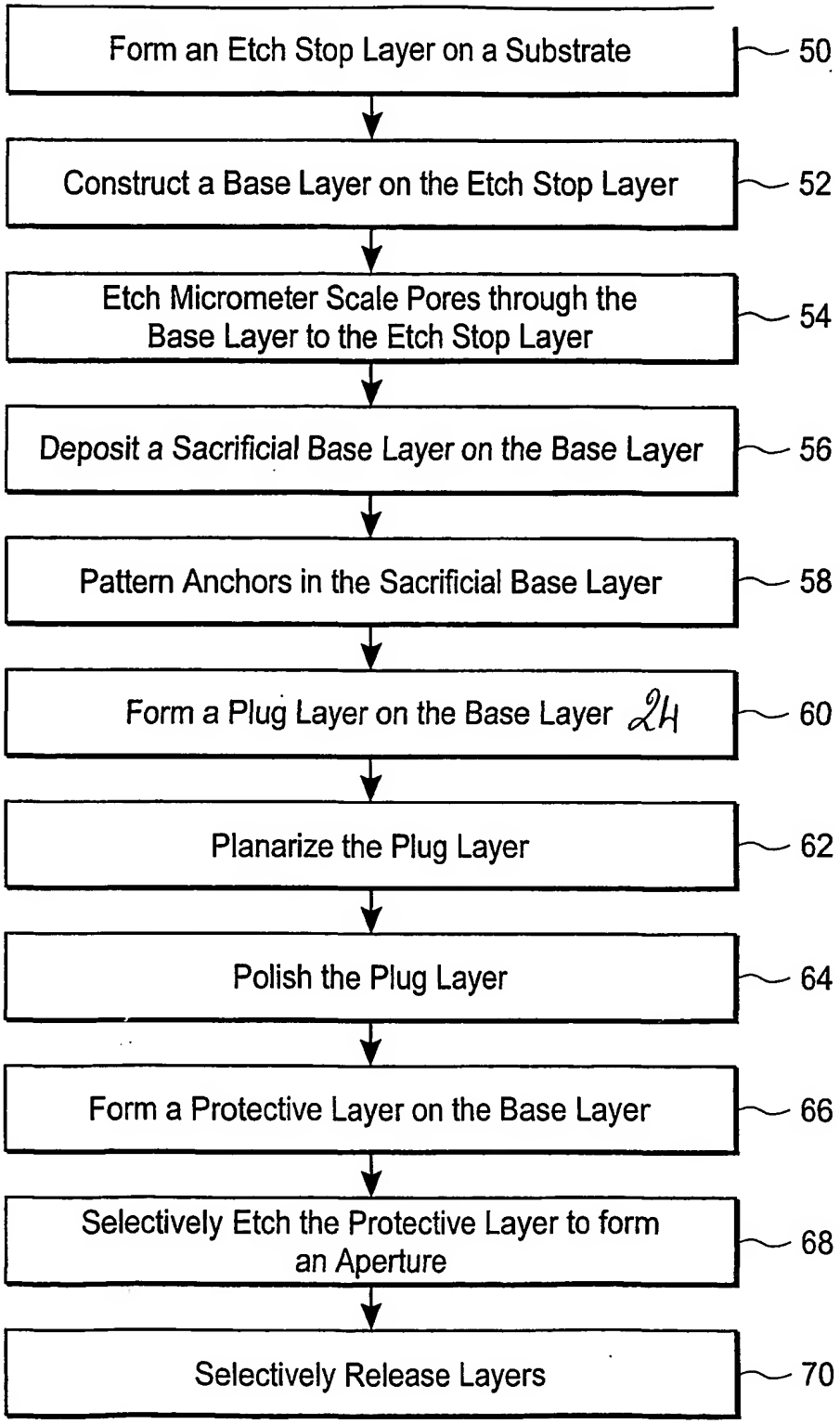


FIG. 10

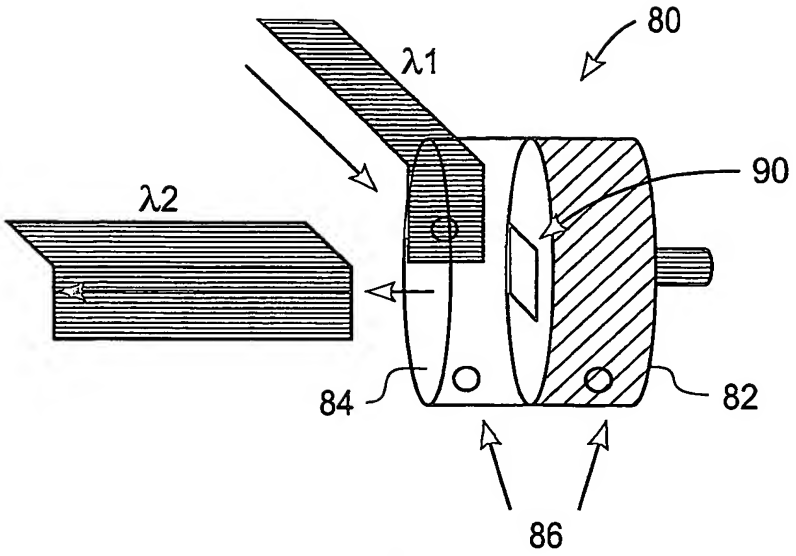


FIG. 11

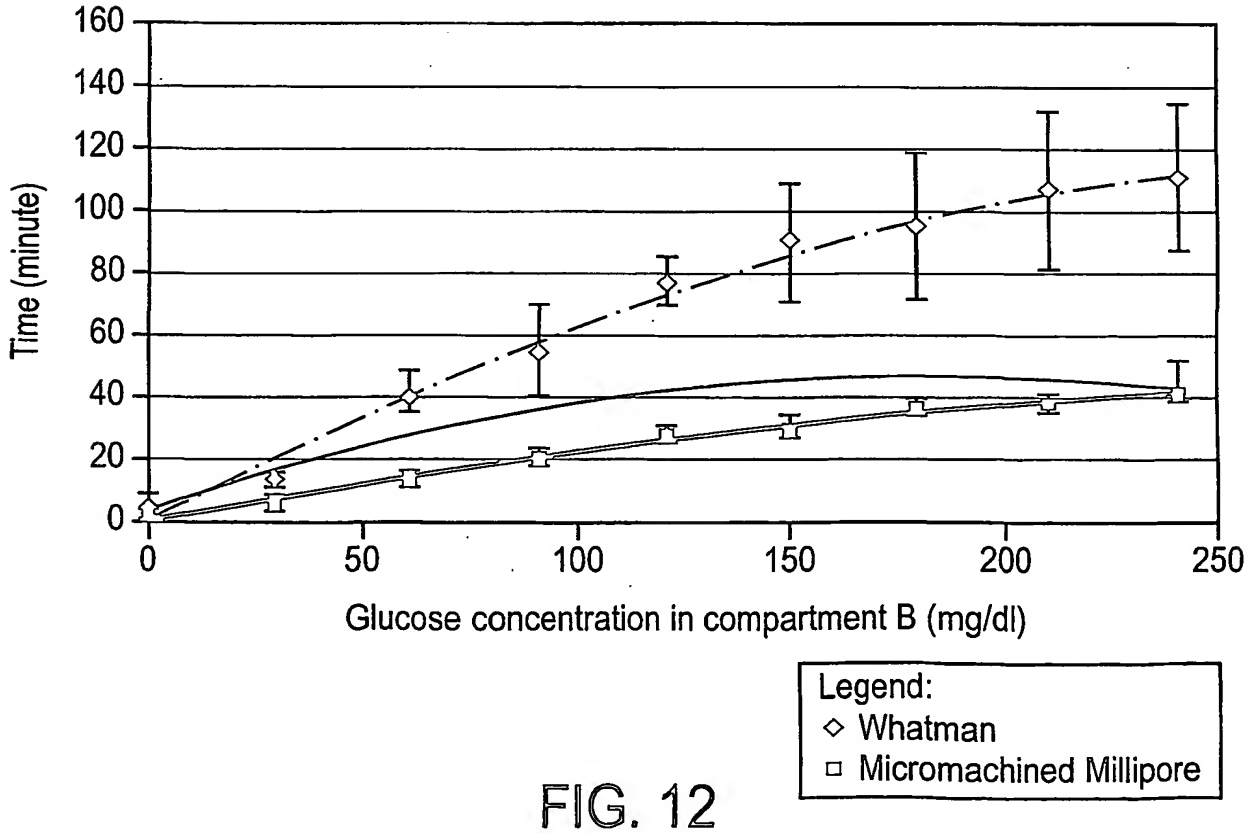


FIG. 12

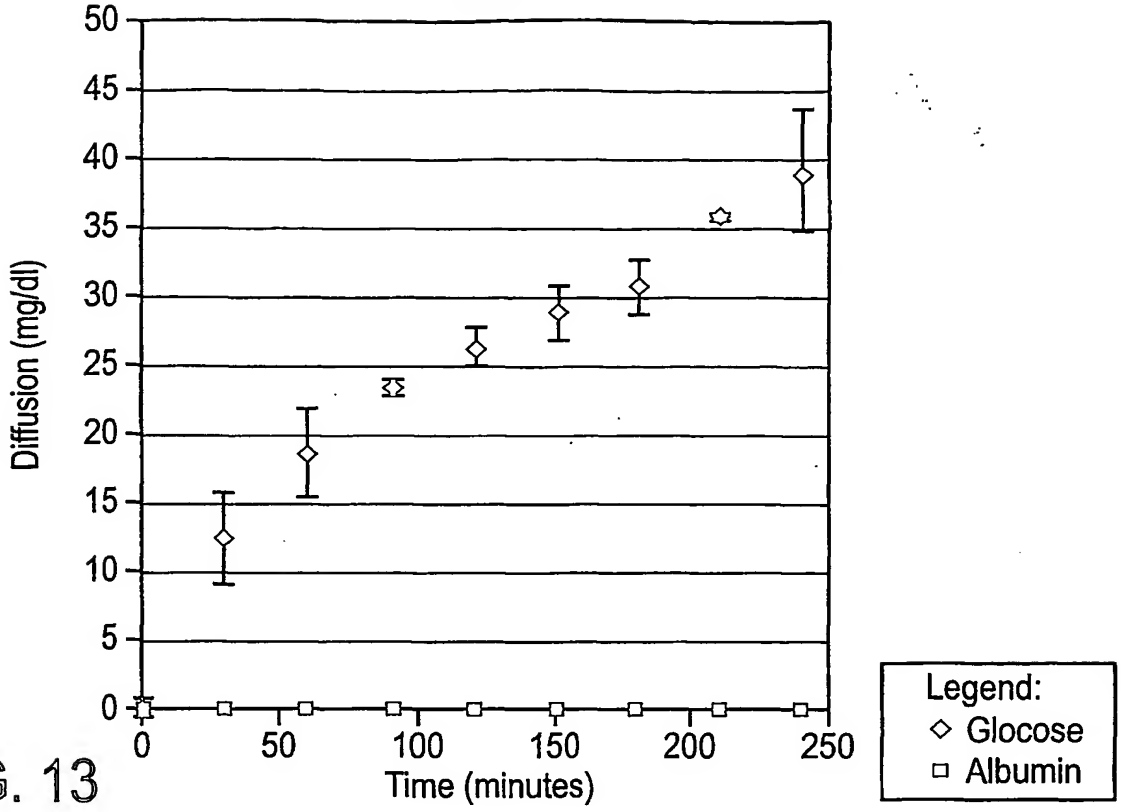


FIG. 13

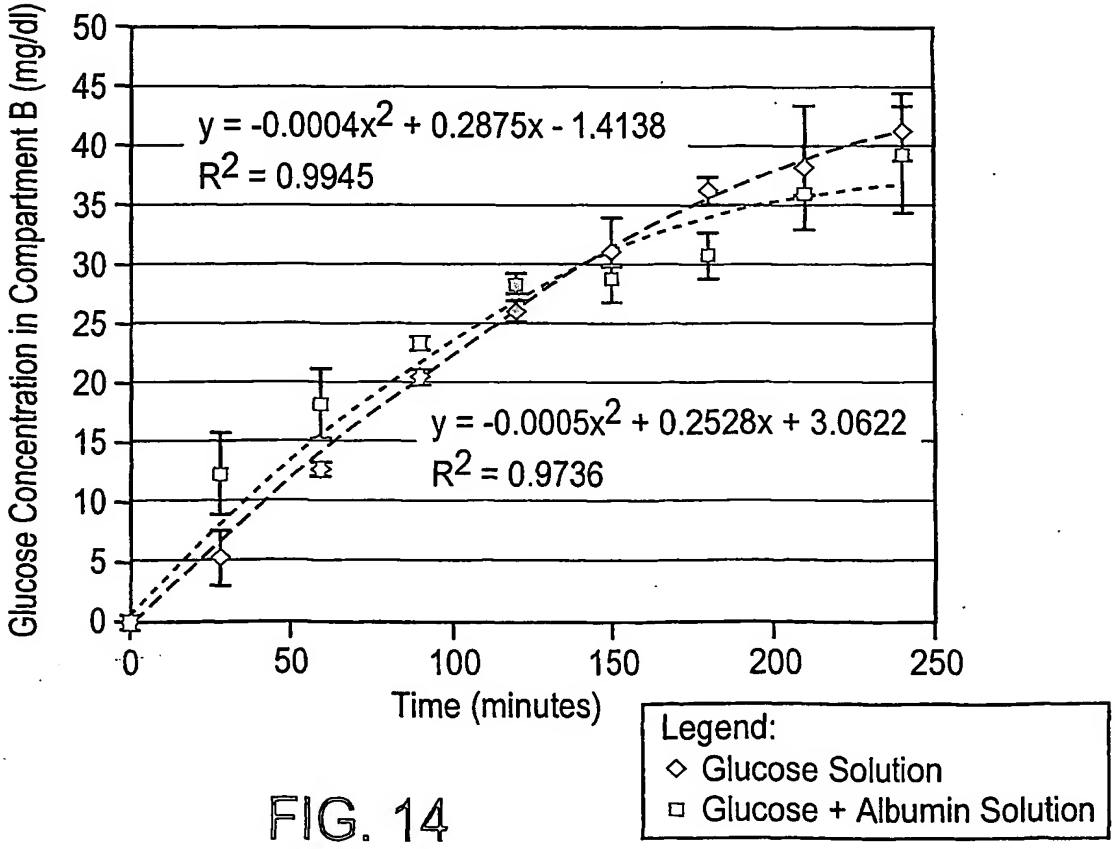


FIG. 14

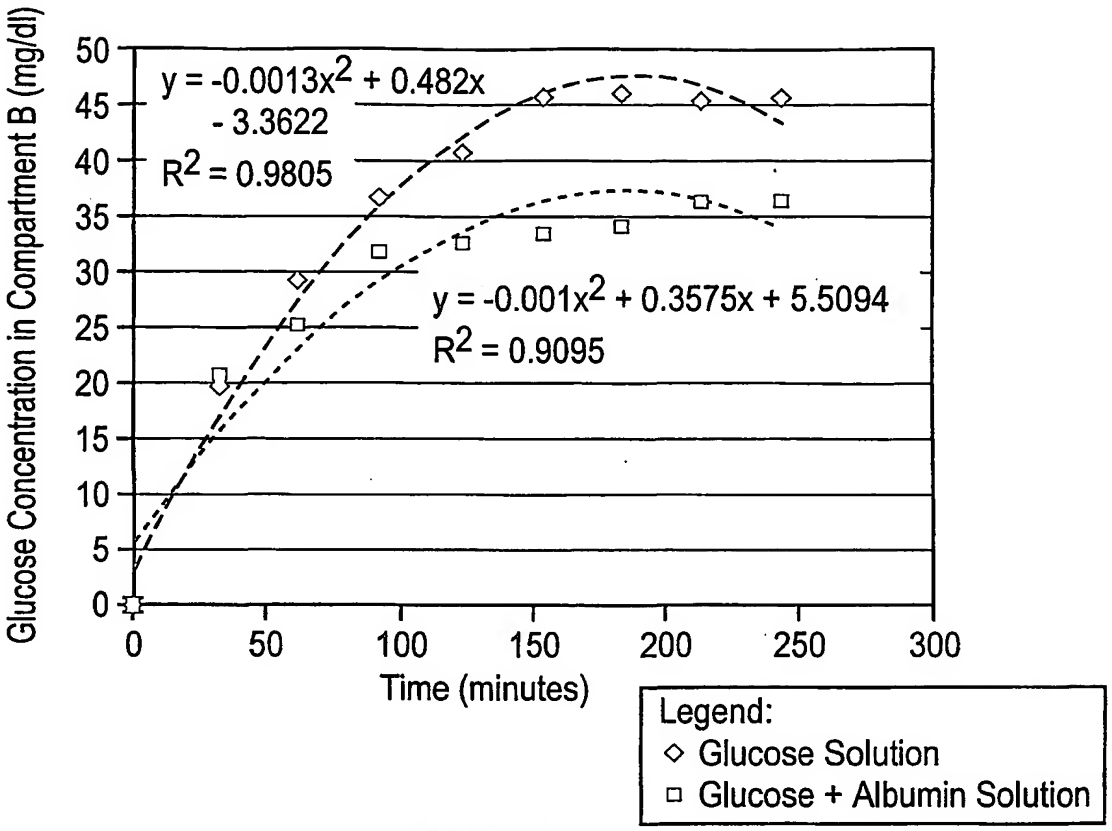


FIG. 15

	WHATMAN		MILLIPORE		MICROMACHINED	
TIME	ALBUMIN CONC.		ALBUMIN CONC.		ALBUMIN CONC.	
Minutes	ABS.	g/dL	ABS.	g/dL	ABS.	g/dL
0	0.381	3.88	0.423	4.31	0.395	3.980
420	0.352	3.58	0.398	4.05	0.394	3.970
Amount of Albumin diffused		0.3 g/dL		0.26 g/dL		0.01 g/dL

FIG. 16

INTERNATIONAL SEARCH REPORT

Intern: ıal application No.
PCT/US00/31749

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : B81C 1/00; B82B 1/00, 3/00; B01D 63/00
US CL : 216/2, 56, 88, 89; 10/321.84, 323.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 216/2, 56, 88, 89; 10/321.84, 323.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
none

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,919,364 A (LEBOUITZ et al) 06 July 1999 (06.07.1999), Fig.4-11, column 4, line 60-column 6, line 10.	1-19
Y	US 5,948,255 A (KELLER et al) 07 September 1999 (07.09.1999), Fig.1A-1G, column 3, line 66-column 5, line 20.	1-4, 13-19
Y	US 5,770,076 A (CHU et al) 23 June 1998 (23.06.1998), columns 4-8.	1-19
Y	US 5,798,042 A (CHU et al) 25 August 1998 (25.08.1998), abstract	1-19
Y	US 5,938,923 A (TU et al) 17 August 1999 (17.08.1999), abstract.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2001 (28.01.2001)	21 MAR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Anita Alanko Telephone No. 703-308-0661

Form PCT/ISA/210 (second sheet) (July 1998)